Baseline Monitoring of Codling Moth (Lepidoptera: Tortricidae) Larval Response to Benzoylhydrazine Insecticides

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ABSTRACT A diet-incorporation larval bioassay was developed to measure the response of codling moth, *Cydia pomonella* (L.), to the benzoylhydrazine insecticides tebufenozide and methoxyfenozide. The bioassay tested neonates and third, fourth, and fifth instars from a laboratory colony and neonates and fourth instars from a pooled population collected from five certified-organic apple orchards. Bioassays were scored after 6 and 14 d. No differences between the laboratory and field population were found for either insecticide. Significant differences were found in the response of third and fifth instars between the 6 and 14 d bioassays, primarily due to a high proportion of moribund larvae in the shorter assay. Larval age had a significant effect in bioassays and was more pronounced in 6- versus 14-d tests. Fifth instars were significantly less susceptible to both insecticides than other stages, while responses of third and fourth instars were similar. The response of neonates was significantly different from third and fourth instars to tebufenozide but not with methoxyfenozide in the 14-d test. Field bioassays excluded the use of fifth instars and were scored after 14 d. LC_{50}s estimated for 18 field-collected populations varied five- and ninefold for tebufenozide and methoxyfenozide, respectively. The responses of all but six field-collected populations were significantly different from the laboratory strain. Five of these six populations were collected from orchards with no history of organophosphate insecticide use. The LC_{50} for methoxyfenozide of one field-collected population reared in the laboratory for three generations declined fourfold, but was still significantly different from the laboratory population. These data suggest that transforming current codling moth management programs in Washington from a reliance on organophosphate insecticides to benzoylhydrazines may be difficult.

KEY WORDS *Cydia pomonella*, insecticide bioassay, insecticide resistance, tebufenozide, methoxyfenozide

INSECTICIDE RESISTANCE HAS been documented in codling moth, *Cydia pomonella* (L.), to all currently used major classes of insecticides: organophosphates (Bush et al. 1993, Varela et al. 1993, Knight et al. 1994, Dunley and Welter 2000), synthetic pyrethroids (Sauphanor et al. 1998, Dunley and Welter 2000), benzoylureas (Moffit et al. 1988, Riedl and Zelger 1994, Sauphanor et al. 1994), and juvenile hormone analogs (Sauphanor and Bouvier 1995; Dunley and Welter 2000). The occurrence of cross-resistance to several classes of insecticides in some populations of codling moth is a dire threat for pome fruit pest management (Sauphanor and Bouvier 1995, Dunley and Welter 2000). This threat is compounded by the continued loss of insecticides due to regulatory actions. For example, implementation of the Food Quality Protection Act (FQPA) of 1996 has already led to the loss of methyl parathion and has further restricted the use of other pesticides in the United States. Additional restrictions and losses are likely to occur before FQPA is fully implemented (Whalon et al. 1999).

In 1999, tebufenozide, a benzoylhydrazine insecticide, became the first registered insect growth regulator for codling moth control on apple, *Malus domestica* (Borkhausen), in the United States. The benzoylhydrazine insecticides are ecdysone agonists that have a broad level of activity for lepidopteran species (Dhadialla et al. 1998). Although the primary mode of action of benzoylhydrazines for codling moth is against the larval stage, these materials are also ovicidal (Pons et al. 1999; A.L.K., unpublished data) and effect adult female fecundity, egg fertility, and spermatogenesis (Friedländer and Brown 1995, Sun and Barrett 1999; A.L.K., unpublished data). Benzoylhydrazines have low toxicity for the major classes of generalist predators and parasitoids within orchards and may become an important, selective tool for tree fruit pest management (Valentine et al. 1996).

However, resistance to this class of insecticides by several tortricid pest species before its field use in apple has already been reported. Sauphanor and Bouvier (1995) found high levels (20-fold) of benzoylhydrazine resistance in a diflubenzuron-resistant laboratory strain of codling moth originally collected in

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southern France. However, there was scant evidence for cross-resistance between azinphosmethyl and tebufenozide (Sauphanor and Bouvier 1995). Sauphanor et al. (1998) later reported that only low levels of resistance to azinphosmethyl (less than or equal to threefold) developed in a field-collected codling moth strain after seven generations of selection with either deltamethrin, diflubenzuron, or diflubenzuron and phosalone. In comparison, the deltamethrin-selected population exhibited a 44-fold level of resistance to tebufenozide. Biddinger et al. (1996) did not detect cross-resistance with tebufenozide in a organophosphate-resistant laboratory strain of *Platynota idaeusalis* (Walker). However, field studies in New York with *Choristoneura rosaceana* (Harris), in Washington with *C. rosaceana* and *Pandemis pyrusana* Kearfott, and in New Zealand with *Planotortrix octo* Dugdale have all reported cross-resistance between azinphosmethyl and either tebufenozide or methoxyfenozide (Wearing 1998, Waldstein et al. 1999; J.E.D., unpublished data).

The potential for cross-resistance is of concern in the United States where the benzoylhydrazine insecticides have been suggested as replacements for organophosphate insecticides (Dunley and Welter 2000). These findings highlight the importance of developing and implementing resistance management strategies concurrently with the registration of new insecticides. The objective of our study was to develop a field-based bioassay to measure codling moth larval response to the benzoylhydrazine insecticides. The response of neonates versus later larval instars was compared to evaluate the use of this bioassay in either testing field-collected larvae or their offspring following laboratory rearing. This bioassay was then used to establish the baseline level of susceptibility of codling moth populations before the use of these insecticides in apple pest management in Washington State.

Materials and Methods

Bioassay Development. Larval bioassay methods were developed with both a laboratory population and offspring from diapausing larvae collected from five certified-organic apple orchards in 1997. The laboratory strain has been maintained for over 25 yr at the USDA-ARS Laboratory in Yakima, WA, and is reared on a soybean-wheat germ diet (Toba and Howell 1991). Field strains were established by placing the adults reared from field-collected larvae in 500-ml clear plastic containers in an outdoor-screened building, and moths were provided with both water and a 10.0% honey solution wick. Bioassays were first conducted with neonates collected from the F_1 generation of each population. Because the bioassay results were not significantly different among the five populations (A.L.K., unpublished data) these data were pooled and are presented as a composite strain. Subsequently, F_1 larvae from these five populations were reared together on the soybean-wheat germ diet and bioassays of fourth instars were conducted with F_2 larvae from the combined population (designated as the 'organic' population hereafter).

Larval bioassays were conducted with a premixed soybean-wheat germ diet (Stonefly Industries, Bryan, TX) impregnated with tebufenozide or methoxyfenozide (Rohm and Haas, Spring House, PA). Batches of diet were mixed in plastic containers (250 ml) by adding 50 g of dry material with 150 ml distilled water. Four to six concentrations plus a nontreated control were included in each bioassay, and groups of 10 larvae were treated as a replicate. One to 10 replicates were conducted with each population and insecticide combination. Concentrations of tebufenozide ranged from 1.0 to 30.0 ppm, and concentrations of methoxyfenozide ranged from 0.03 to 3.0 ppm in these tests. The mixed diet was spooned into plastic cups (30-ml), and a small piece of corrugated cardboard was glued to the inside of the lid of the cup to provide a pupation site. Individual larvae were placed in each cup and maintained at 25°C and a photoperiod of 16:8 (L:D) h. Larvae not responding with head movement or peristaltic contractions when probed with a fine brush were scored as dead. Moribund larvae were recorded as alive. Missing larvae in the neonate assays were counted as dead (<10.0%). In some trials, older larvae chewed their way out of the cup and these larvae were scored as alive (<10.0%). Cup lids were wrapped on the outside with aluminum foil to reduce larval escape in some later assays. Control mortality did not exceed 10% during the bioassays.

Several series of tests were conducted to refine this bioassay method. Bioassays were conducted to compare the response of larvae in 6 d versus 14 d tests, to compare larvae from the laboratory versus the pooled field-collected strain, and to compare the responses of neonates and third, fourth, and fifth instars to both insecticides. Larvae were removed from pans of diet and grouped as third, fourth, or fifth instars based on head capsule measurements (Weitzner and Whalon 1987). The mean (±SE) weights of each of the three instars from the laboratory strain were 6.2 (0.3), 13.4 (0.4), and 24.5 (0.7) mg, respectively. The mean (±SE) weight of fourth instars from the organic strain was 12.8 (0.6) mg. Neonates (<24 h old) for both strains were collected from waxed paper oviposition sheets previously placed in the adult mating containers. Cohorts of laboratory-reared third and fifth instars were independently tested in 6- and 14-d bioassays. Bioassays of neonates and fourth instars were checked at both 6 and 14 d.

Baseline Studies of Field Populations. Codling moth larvae infesting apple fruits were collected from a variety of sites during 1998 and 1999, including certified organic orchards, unmanaged sites such as backyard trees and unsprayed research plots, and conventionally managed orchards. In most cases, conventional orchards were treated with sex pheromone dispensers for mating disruption of codling moth and were also treated with zero to two organophosphate insecticide applications during the year that the infested fruit were collected. All fruit were maintained in the laboratory at 5°C before the bioassay. Apples were cut to remove the larvae, and

Table 1. Comparison of the effect of bioassay length on the response of third- and fifth-instar codling moth from a laboratory colony in a diet-incorporation bioassay with tebufenozide and methoxyfenozide

Insecticide	Larval stage	Bioassay length, d								
		6				14				
		n	Slope (SE)	LC_{50} ppm (95% FL)	χ^{2a}	*n*	Slope (SE)	LC_{50} ppm (95% FL)	χ^{2a}	LCR[b] (95% CL)
Tebufenozide	3rd instar	169	2.96 (0.46)	2.58 (1.86–3.68)	24.6	150	6.54 (0.95)	1.42 (1.24–1.65)	10.4	1.82* (1.39–2.37)
Tebufenozide	5th instar	379	1.32 (0.16)	5.74 (4.36–7.63)	53.3*	118	4.93 (0.78)	1.94 (1.62–2.30)	9.9	2.96* (2.24–3.92)
Methoxyfenozide	3rd instar	293	2.69 (0.59)	0.49 (0.36–0.62)	19.6	130	2.32 (0.45)	0.12 (0.07–0.16)	12.0	4.21* (2.76–6.44)
Methoxyfenozide	5th instar	313	1.15 (0.23)	0.93 (0.57–2.08)	33.0	120	4.90 (0.80)	0.22 (0.18–0.26)	9.2	4.28* (2.44–7.52)

[a] Asterisk denotes that the data do not fit the probit model at $P < 0.05$, chi-square goodness-of-fit test.
[b] LCR, lethal concentration ratio equals LC_{50} (6 d)/LC_{50} (14 d). * Indicates a significant difference $P < 0.05$ (Robertson and Priesler 1992).

larvae injured during the cutting of the fruit were discarded. Fifth instars (>20.0 mg) collected from infested fruit were not included in these studies.

Statistical Analyses. Dose-mortality regressions were calculated with both insecticides using the probit option of POLO (LeOra Software 1987). Lethal concentration ratios (LCRs) of LC_{50}s were calculated to compare the responses of neonates and fourth instars for the laboratory and pooled organic strains; and to evaluate the effect of larval age (neonates, third, fourth, and fifth instars) for the laboratory strain (Robertson and Preisler 1992). Lethal concentration ratios were also computed to compare the responses of field-collected populations with fourth instars and neonates of the laboratory strain. Ratios with 95% CL not including 1.0 were considered significantly different, $P < 0.05$ (Robertson and Preisler 1992). Correlation statistics were computed to compare the LC_{50}s of populations exposed to both insecticides across years.

Results

Bioassay Development. The length of the bioassay was an important factor affecting larval response to each insecticide (Table 1). The responses of third and fifth instars differed at 6 and 14 d. LC_{50}s declined 45–66% and 76–77% from the 6-d to the 14-d bioassay for tebufenozide and methoxyfenozide, respectively. These differences in larval mortality between time periods was due to the high proportion of moribund larvae scored as alive in the 6-d bioassay. The responses of neonates and fourth instar from the laboratory and organic strains to both tebufenozide and methoxyfenozide did not differ at either 6 or 14 d (Table 2).

Larval age was a significant factor affecting the response of codling moth in these bioassays (Table 3). Fifth instars were significantly less susceptible to both insecticides than all other larval stages at both time intervals. The response of neonates to tebufenozide

Table 2. A comparison of the responses of codling moth larvae from a laboratory colony and a population pooled from five certified organic apple orchards during 1997 in diet-incorporation bioassays with tebufenozide and methoxyfenozide

Insecticide	Test duration, d	Larval stage	Laboratory				Organic				LCR[b] (95% CL)
			n	Slope (SE)	LC_{50} ppm (95% FL)	χ^{2a}	*n*	Slope (SE)	LC_{50} ppm (95% FL)	χ^{2a}	
Tebufenozide	6	Neonate	302	9.21 (1.12)	1.39 (1.05–1.67)	114.1*	550	7.20 (0.57)	1.42 (1.26–1.58)	138.9*	1.02 (0.91–1.15)
Tebufenozide	6	4th instar	397	1.32 (0.16)	2.52 (1.60–3.62)	62.0*	220	4.04 (0.67)	2.87 (2.35–3.92)	46.9*	1.12 (0.81–1.54)
Tebufenozide	14	Neonate	120	5.33 (0.99)	1.03 (0.77–1.29)	16.8	550	7.88 (0.76)	1.19 (0.98–1.41)	293.8*	1.15 (0.96–1.37)
Tebufenozide	14	4th instar	110	6.28 (1.02)	1.46 (1.26–1.74)	5.4	220	5.58 (0.67)	1.47 (1.20–1.71)	39.7*	1.01 (0.83–1.22)
Methoxyfenozide	6	Neonate	277	3.73 (0.58)	0.19 (0.15–0.23)	21.9	520	5.32 (0.51)	0.16 (0.14–0.18)	65.5	0.84 (0.68–1.06)
Methoxyfenozide	6	4th instar	246	2.16 (0.32)	0.37 (0.27–0.52)	30.4	180	1.49 (0.20)	0.47 (0.29–0.83)	22.7	1.27 (0.81–2.00)
Methoxyfenozide	14	Neonate	125	3.14 (0.46)	0.15 (0.10–0.25)	26.7*	600	5.50 (0.87)	0.11 (0.10–0.12)	47.4	0.82 (0.65–1.04)
Methoxyfenozide	14	4th instar	120	2.75 (0.53)	0.13 (0.09–0.16)	9.8	239	1.65 (0.28)	0.14 (0.07–0.21)	22.6	1.06 (0.65–1.72)

[a] Asterisk denotes that the data do not fit the probit model at $P < 0.05$, chi-square goodness-of-fit test.
[b] LCR, lethal concentration ratio equals LC_{50} (organic)/LC_{50} (laboratory). No significant differences ($P < 0.05$) were detected between populations (Robertson and Priesler 1992).

Table 3. Comparisons of responses (lethal concentration ratios [LCR] computed from LC_{50}s) among larval stages for a laboratory strain of codling moth to tebufenozide and methoxyfenozide in 6-d and 14-d diet incorporation bioassays

Insecticide	Instar	LCR (95% CL)[a]					
		6 d			14 d		
		Neonate	3rd instar	4th instar	Neonate	3rd instar	4th instar
Tebufenozide	3rd	1.85* (1.45–2.38)	—	—	1.37* (1.11–1.70)	—	—
Tebufenozide	4th	1.81* (1.32–2.48)	0.98 (0.67–1.42)	—	1.41* (1.13–1.77)	1.03 (0.84–1.27)	—
Tebufenozide	5th	4.13* (3.24–5.25)	2.22* (1.62–3.05)	2.27* (1.56–3.33)	1.87* (1.48–2.37)	1.36* (1.10–1.70)	1.32* (1.05–1.66)
Methoxyfenozide	3rd	2.58* (1.88–3.54)	—	—	0.85 (0.57–1.27)	—	—
Methoxyfenozide	4th	1.97* (1.43–2.72)	0.76 (0.53–1.09)	—	0.93 (0.66–1.33)	1.10 (0.71–1.71)	—
Methoxyfenozide	5th	4.93* (2.79–8.72)	1.91* (1.06–3.45)	2.50* (1.38–4.55)	1.60* (1.21–2.11)	1.88* (1.28–2.76)	1.72* (1.23–2.38)

[a] LCR, lethal concentration ratio equals LC_{50} (instar)/LC_{50} (larval stage) computed with data in Tables 1 and 2; * indicates a significant difference $P < 0.05$ (Robertson and Priesler 1992).

was also significantly different from all other larval stages at both time intervals. However, the response of neonates to methoxyfenozide was significantly different from all other larval stages only in the 6-d bioassay. In contrast, the response of neonates in the 14-d bioassay was only significantly different from fifth instars (Table 3). The responses of third instars and fourth instars did not differ in bioassays with either insecticide measured at each period.

Baseline Studies of Field Populations. Significant differences were found in the response of F_1 neonates among four field populations and the laboratory strain (Table 4). The Garza population in both years and the Walla population in 1999 were significantly different from the laboratory strain to one or both insecticides. Garza was a transitional orchard that had a history of one to two applications of organophosphate insecticides before 1998. Its responses to both insecticides were similar during 1998 and 1999 (Table 4). The Walla orchard was the only conventional orchard population evaluated with the neonate bioassay, and historically had been treated with several organophosphate insecticide sprays each year. However, during 1996 and again in 1999, portions of this orchard were treated experimentally with tebufenozide and methoxyfenozide. Overwintering larvae were collected from this orchard in 1998, but oviposition by this population was low and tests were not conducted with neonates. Two populations, Hood and Gfuji, were collected from organic orchards, and their responses were not significantly different from the laboratory strain (Table 4).

Bioassays were conducted with 16 populations of codling moth collected as larvae from infested fruit (Table 5). LC_{50}s calculated from these population's responses to tebufenozide and methoxyfenozide varied five- and ninefold, respectively (Table 5). Correlations of the responses of the four populations bioassayed with both insecticides during 1998 and 1999 (Table 5) were not significant (LC_{50}s: $r = 0.85$, df = 3, $P = 0.15$).

Five of the seven populations assayed with tebufenozide had significantly higher LC_{50}s than the laboratory strain (Table 5). Four out of five of these orchards were conventionally managed and received spray applications of organophosphate insecticides. Brads population was an unsprayed backyard tree situated within city limits of Yakima, WA, and >2.0 km from the nearest commercial tree fruit production. The Redm population was the most susceptible population collected from a conventional orchard. The codling moth-infested fruit in this orchard were only

Table 4. The responses to tebufenozide and methoxyfenozide of F_1 neonates reared from a parent colony of codling moth collected as larvae from infested fruit in apple orchards under variable management during 1998–1999

Population	Management[a]	Insecticide	n	Slope (SE)	LC_{50} ppm (95% FL)	χ^{2b}	LCR[c] (95% CL)
Hood-98	Organic	Tebufenozide	40	4.63 (1.62)	1.17 (0.60–1.56)	0.6	1.13 (0.79–1.63)
Garza-98	Organic	Tebufenozide	40	3.98 (1.25)	1.94 (1.30–2.54)	0.7	1.88 (1.38–2.56)*
Garza-99	Organic	Tebufenozide	80	3.07 (0.62)	2.73 (1.81–4.95)	9.0	2.64 (1.94–3.59)*
Gfuji-98	Organic	Methoxyfenozide	70	2.32 (0.71)	0.09 (0.03–0.14)	0.7	0.67 (0.36–1.25)
Garza-98	Organic	Methoxyfenozide	115	2.77 (0.48)	0.21 (0.12–0.30)	29.5	1.53 (1.09–2.14)*
Garza-99	Organic	Methoxyfenozide	175	2.87 (0.52)	0.28 (0.18–0.38)	23.5	2.06 (1.45–2.93)*
Walla-99	Conv.	Methoxyfenozide	166	4.00 (0.73)	0.45 (0.37–0.55)	11.5	3.34 (2.51–4.44)*

[a] Management of orchards included certified organic and conventional (Conv.) insecticide programs.
[b] No chi-square values significant at $P = 0.05$ level.
[c] LCR, lethal concentration ratio equals LC_{50} (field-collected population)/LC_{50} (laboratory strain neonates, 14 d bioassay [Table 2]); * indicates a significant difference $P < 0.05$ (Robertson and Priesler 1992).

Table 5. The responses of codling moth larvae collected from infested apple fruits in 14-d bioassays with tebufenozide and methoxyfenozide during 1998 and 1999

Population	Management[a]	Insecticide	*n*	Slope (SE)	LC_{50} ppm (95% FL)	χ^{2b}	LCR[c] (95% CL)
Redm-98	Conv.	Tebufenozide	40	3.98 (1.47)	1.45 (0.86–2.12)	1.5	0.99 (0.63–1.56)
Garza-98	Organic	Tebufenozide	190	1.62 (0.28)	1.95 (0.81–3.29)	32.3*	1.34 (0.87–2.05)
Brads-98	Unmanaged	Tebufenozide	140	1.67 (0.28)	3.29 (1.76–6.37)	21.1	2.43 (1.61–3.69)*
Pbloc-99	Conv.	Tebufenozide	40	3.66 (0.95)	4.06 (2.89–6.59)	0.6	2.78 (1.88–4.10)*
Basin-99	Conv.	Tebufenozide	40	4.31 (1.11)	4.68 (3.37–7.40)	1.3	3.20 (2.19–4.67)*
Case-99	Conv.	Tebufenozide	114	2.16 (0.38)	5.69 (3.62–11.54)	19.0*	3.89 (2.74–5.51)*
Walla-99	Conv.	Tebufenozide	200	1.48 (0.27)	7.29 (4.44–23.81)	37.2*	4.99 (3.17–7.84)*
Sulli-99	Unmanaged	Methoxyfenozide	100	1.58 (0.30)	0.17 (0.08–0.37)	14.3	1.30 (0.79–2.12)
TFREC-98	Unmanaged	Methoxyfenozide	47	3.79 (1.26)	0.20 (0.10–0.27)	0.3	1.54 (0.98–2.43)
Garza-98	Organic	Methoxyfenozide	307	1.70 (0.20)	0.27 (0.17–0.39)	31.5	2.12 (1.42–3.15)*
Walla-99 F_3	Conv.	Methoxyfenozide	100	1.83 (0.34)	0.33 (0.09–0.65)	28.7	2.60 (1.60–4.23)*
Redm-98	Conv.	Methoxyfenozide	78	0.87 (0.30)	0.35 (0.07–0.91)	5.7	2.78 (1.03–7.47)*
Garzaw-99	Organic	Methoxyfenozide	100	1.87 (0.21)	0.52 (0.18–1.30)	12.5	4.10 (2.11–7.96)*
Walla-99 F_2	Conv.	Methoxyfenozide	120	3.62 (0.68)	0.61 (0.34–1.00)	25.3*	4.82 (3.11–7.45)*
Chero-99	Conv.	Methoxyfenozide	100	0.89 (0.18)	0.55 (0.14–1.62)	11.5	4.34 (1.98–9.50)*
Night-99	Conv.	Methoxyfenozide	80	6.45 (2.04)	0.57 (0.40–0.72)	2.9	4.50 (3.13–6.47)*
Stolen-99	Conv.	Methoxyfenozide	100	2.32 (0.43)	0.62 (0.39–0.95)	9.8	4.87 (3.22–7.37)*
Garza-99	Organic	Methoxyfenozide	80	1.24 (0.18)	0.74 (0.44–1.54)	2.6	5.83 (3.14–10.82)*
Fossu-99	Conv.	Methoxyfenozide	100	2.06 (0.36)	0.86 (0.52–1.47)	11.0	6.76 (4.36–10.48)*
Smith-99	Conv.	Methoxyfenozide	100	1.48 (0.24)	1.01 (0.23–4.31)	41.6*	7.96 (4.51–14.04)*
Walla-99 F_1	Conv.	Methoxyfenozide	130	1.68 (0.25)	1.22 (0.73–2.43)	17.6	9.61 (5.98–15.46)*
Frost-99	Conv.	Methoxyfenozide	50	1.15 (0.29)	1.28 (0.52–3.45)	3.0	10.03 (4.16–24.20)*
Walla-98	Conv.	Methoxyfenozide	158	1.12 (0.30)	1.3 (0.60–4.16)	18.9	10.33 (5.08–21.00)*
Walla-99	Conv.	Methoxyfenozide	300	1.19 (0.15)	1.41 (0.71–2.33)	68.6*	11.08 (7.16–17.15)*
Pbloc-99	Conv.	Methoxyfenozide	90	1.28 (0.25)	1.45 (0.58–10.02)	12.0	11.37 (5.49–23.58)*

[a] Orchards were managed with certified organic or conventional insecticide programs, or were unmanaged.
[b] Asterisk denotes that the data does not fit the probit model at $P < 0.05$, chi-square goodness-of-fit test.
[c] LCR, lethal concentration ratio equals LC_{50} (field-collected population)/LC_{50} (laboratory strain 4th instar, 14 d-bioassay); * indicates a significant difference $P < 0.05$ (Robertson and Priesler 1992).

collected on the first three rows of the orchard next to a newly stacked bin pile. This orchard did not have a history of codling moth, and this localized infestation was likely due to moths emerging from the bin pile. The history of insecticide use of the orchard(s) where these bins had been used in 1997 is unknown.

All but two of the 13 populations bioassayed with methoxyfenozide had significantly higher LC_{50}s than the laboratory population (Table 5). The two susceptible populations were both from unmanaged orchards, Sulli and TFREC. The responses of populations collected from the organic orchards, Garzaw and Garza, were both significantly different from the laboratory strain. The responses to methoxyfenozide of all codling moth populations from conventional orchards were significantly different from the laboratory strain (Table 5). The Pbloc orchard had the highest LC_{50} to methoxyfenozide. The insecticide use history of the Pbloc is unknown because it has been used exclusively for the testing of experimental pesticides during the past decade.

The Walla populations bioassayed in both 1998 and 1999 were significantly more tolerant to methoxyfenozide than the laboratory population (Table 5). A large number of infested fruit were collected from this orchard in 1999, and a population was established in the laboratory on artificial diet. The LC_{50} of the Walla-99 population to methoxyfenozide declined after each generation (Table 5). After three generations the LC_{50} was fourfold lower than the parent generation; however, its response was still significantly different from the laboratory strain (Table 5).

Discussion

Larval age and length of exposure were significant factors affecting the response of codling moth to tebufenozide and methoxyfenozide. Benzoylhydrazine insecticides interfere with the insect molting process by substituting for the insect molting hormone, 20-hydroxy ecdysone, and preventing the release of the occlusion hormone (reviewed in Dhadialla et al. 1998). Toxic symptoms in exposed larvae typically involve a cessation of feeding within 4–16 h and the slippage of the head capsule (Wing et al. 1988). Larval death is caused by starvation and dehydration, and the length of survivorship following exposure is likely determined by the size of the larva. The presence of moribund larvae in bioassays after 6 d reduced our ability to measure insecticide toxicity. Extension of the bioassay to 14 d and the exclusion of fifth instars made this bioassay a more practical tool to measure responses of field populations.

A number of factors are important in developing an appropriate larval bioassay technique for codling moth. Larval studies of codling moth field populations can be accomplished by either collecting larvae from infested fruit or rearing adults and testing F_1 offspring (neonates or larger larvae). Both methods have certain disadvantages. Drawbacks of bioassays using field-collected larvae are that codling moth populations generally occur at low densities in orchards and that measurable populations can only be collected from specific sites. Heavy codling moth infestations were located in either unmanaged sites, certified organic

orchards, or conventional orchards treated with sex pheromones and limited use of organophosphate insecticides. This subset of orchards is likely not representative of the apple industry in Washington. We presume that codling moth was a serious pest in the conventional orchards included in our study due to the ineffective use of insecticides. However, we did not identify these populations' responses to other insecticides, and cannot ascertain whether management problems were due to insecticide resistance or other factors, such as poor spray timing and coverage. Thus, it is possible that the response of codling moth populations from these conventional orchards may not be representative of the low population densities found in the majority of orchards within the region.

A second general problem with bioassays using field-collected populations is the lack of control in the quality of the test subjects from prior exposure to exogenous factors and from transport into the laboratory. The effects of these factors on the response of the field populations we tested were unknown; however, the quality of infested fruit was good and larvae were protected within the interior of chilled fruit before testing. The weight and age of the field-collected third and fourth instars varied substantially in these tests (2.8–21.8 mg). Yet, our data suggest that this variation has a minimal impact on larval survivorship in the 14-d bioassays. In contrast, fifth instars exhibit a significantly different response with a higher proportion able to successfully pupate with no or limited feeding on the insecticide-impregnated diet.

Conducting insecticide bioassays with codling moth using F_1 larvae instead of field-collected larvae may be preferable when overwintering or summer larvae can be collected in corrugated strips placed around the trunks of trees, or when adult moths can be trapped alive. However, there are many problems associated with bioassays requiring laboratory establishment of test populations. Using F_1 larvae requires more handling and rearing of insects. Bioassays of older F_1 larvae require rearing on thinning apples or artificial diet. Oviposition by codling moth populations collected as overwintering larvae in diapause is often low and may preclude the use of standard egg or larval bioassays (Sauphanor and Bouvier 1995). Finally, genetic drift or selection in laboratory-reared populations can cause changes in bioassay response relative to the original parental response, e.g., the response of the Walla-99 population to methoxyfenozide (Table 5).

Results from this study indicate that there is considerable variation present in the direct toxicity of tebufenozide and methoxyfenozide to codling moth populations in Washington State apple orchards. At present, it is not clear whether this variability is due to differing histories of pesticide exposure, an inherent factor due to the experimental method, or portrays the natural variation in the response of codling moth to benzoylhydrazine insecticides. The gradual reversion of the Walla-99 population's response to methoxyfenozide under laboratory conditions is consistent with a hypothesis that prior exposure to pesticide residues may have selected for resistance. Several studies have reported the occurrence of cross-resistance between the benzoylhydrazine and organophosphate insecticides among tortricid orchard pests (Wearing 1998, Waldstein et al. 1999; J.E.D., unpublished data). However, the occurrence of similar cross-resistance for codling moth is not evident in several French populations (Sauphanor and Bouvier 1995, Sauphanor et al. 1998). Similar studies are needed in the western United States to assess whether codling moth populations exhibit cross-resistance between the benzoylhydrazines and other classes of insecticides. Tracking the responses of codling moth field populations removed from exposure to organophosphate insecticides by the substitute use of a variety of selective tactics, such as mating disruption, microbial insecticides, and cultural practices will be useful.

Our study examined only the direct toxic effects of tebufenozide and methoxyfenozide to codling moth larvae. Yet, Biddinger and Hull (1999) demonstrated that sublethal larval exposure to tebufenozide reduced adult fecundity in an insecticide-susceptible population of *P. idaeusalis.* Reproductive effects following adult exposure of codling moth have been well documented in laboratory strains and are thought to be an important factor impacting its management in orchards (Pons et al. 1999, Sun and Barrett 1999; A.L.K., unpublished data). Thus, it would be interesting to also survey the phenotypic variation in reproductive effects among these codling moth populations.

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